

FORM PTO-1390 (REV. 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				1624-0132P	
INTERNATIONAL APPLICATION NO.		INTERNATIONAL FILING DATE		U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	
PCT/CN00/00010		January 21, 2000		09/913322	
TITLE OF INVENTION		PRIORITY DATE CLAIMED			
NOVEL GYMNEMIC ACID DERIVATIVES, PROCESS FOR THE PREPARATION THEREOF AND USE THEREOF AS MEDICINE		February 11, 1999			
APPLICANT(S) FOR DO/EO/US					
YE, Wencai; DAI, Yue; CONG, Xiaodong; ZHU, Xingxiang; ZHAO, Shouxun					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
1.	<input checked="" type="checkbox"/>	This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.			
2.	<input type="checkbox"/>	This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.			
3.	<input checked="" type="checkbox"/>	This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).			
4.	<input checked="" type="checkbox"/>	The US has been elected by the expiration of 19 months from the priority date (Article 31).			
5.	<input checked="" type="checkbox"/>	A copy of the International Application as filed (35 U.S.C. 371(c)(2))			
	a.	<input type="checkbox"/>	is transmitted herewith (required only if not transmitted by the International Bureau).		
	b.	<input checked="" type="checkbox"/>	has been transmitted by the International Bureau. WO 00/47594		
	c.	<input type="checkbox"/>	is not required, as the application was filed in the United States Receiving Office (RO/US).		
6.	<input checked="" type="checkbox"/>	An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).			
	a.	<input checked="" type="checkbox"/>	is transmitted herewith.		
	b.	<input type="checkbox"/>	has been previously submitted under 35 U.S.C. 154(d)(4)		
7.	<input checked="" type="checkbox"/>	Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).			
	a.	<input type="checkbox"/>	are transmitted herewith (required only if not transmitted by the International Bureau).		
	b.	<input type="checkbox"/>	have been transmitted by the International Bureau.		
	c.	<input type="checkbox"/>	have not been made; however, the time limit for making such amendments has NOT expired.		
	d.	<input checked="" type="checkbox"/>	have not been made and will not be made.		
8.	<input type="checkbox"/>	An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).			
9.	<input type="checkbox"/>	An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).			
10.	<input type="checkbox"/>	An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).			
Items 11. to 20. below concern document(s) or information included:					
11.	<input type="checkbox"/>	An Information Disclosure Statement under 37 CFR 1.97 and 1.98-International Search Report (PCT/ISA/210) w/ documents			
12.	<input type="checkbox"/>	An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.			
13.	<input checked="" type="checkbox"/>	A FIRST preliminary amendment.			
14.	<input type="checkbox"/>	A SECOND or SUBSEQUENT preliminary amendment.			
15.	<input type="checkbox"/>	A substitute specification.			
16.	<input type="checkbox"/>	A change of power of attorney and/or address letter.			
17.	<input type="checkbox"/>	A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.			
18.	<input type="checkbox"/>	A second copy of the published international application under 35 U.S.C. 154(d)(4).			
19.	<input type="checkbox"/>	A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).			
	<input checked="" type="checkbox"/>	Other items or information:			
		1.) Zero (0) sheets of Formal Drawings			

ATTORNEY'S DOCKET NUMBER

1624-0132P

21. ☒ The following fees are submitted:

**BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):**

Neither international preliminary examination fee (37 CFR 1.482)  
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO  
and International Search Report not prepared by the EPO or JPO. . . . . **\$1,000.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO . . . . .	<b>\$860.00</b>
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International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. . . . .	<b>\$710.00</b>
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International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .....	<b>\$690.00</b>
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International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4).....	<b>\$100.00</b>
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**ENTER APPROPRIATE BASIC FEE AMOUNT =**

Surcharge of **\$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total Claims	14 - 20 =	0	X <b>\$18.00</b>
Independent Claims	6 - 3 =	3	X <b>\$80.00</b>
MULTIPLE DEPENDENT CLAIM(S) (if applicable)		None	+ <b>\$270.00</b>

**TOTAL OF ABOVE CALCULATIONS =**

☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.

**SUBTOTAL =**

Processing fee of **\$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). ☐20 ☐30 +

**TOTAL NATIONAL FEE =**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **\$40.00** per property +

**TOTAL FEES ENCLOSED =**

Amount to be: refunded	\$
charged	\$

- a. ☒ A check in the amount of \$ 1370.00 to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account. No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_ to cover the above fees.  
A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:

**Birch, Stewart, Kolasch & Birch, LLP or Customer No. 2292**

**P.O. Box 747**

**Falls Church, VA 22040-0747**

**(703)205-8000**

**Date: August 10, 2001**

James M. Slattery, #28,380

PATENT  
1624-0132P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: YE, Wencai et al. Conf.:  
Int'l. Appl. No.: PCT/CN00/0010  
Appl. No.: New Group:  
Filed: August 10, 2001 Examiner:  
For: NOVEL GYMNEMIC ACID DERIVATIVES, PROCESS FOR  
THE PREPARATION THEREOF AND USE THEREOF AS  
MEDICINE

PRELIMINARY AMENDMENT

**BOX PATENT APPLICATION**

Assistant Commissioner for Patents  
Washington, DC 20231

August 10, 2001

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE TITLE:

Please amend the title to read:

--NOVEL GYMNEMIC ACID DERIVATIVES, PROCESS FOR THE  
PREPARATION THEREOF AND USE THEREOF AS MEDCINE--

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/CN00/00010 which has an International filing date of January

21, 2000, which designated the United States of America and was not published in English.--

**IN THE CLAIMS:**

Please amend the claims as follows:

10. (Amended) A composition of claim 1, which contains Gymnemic Acid derivative of formula I and/or II, wherein based on the weight of the composition, the contents of compounds A,B,C,D,E and is 1.25-2.10% compound A, 0.89-1.50% compound B, 2.40-3.80% compound C, 2.10-3.40% compound D, 2.74-4.60% compound E and 3.24-5.40% compound F.

Please add the following new claim:

--14. (New) A composition of claim 2, which contains Gymnemic Acid derivative of formula I and/or II, wherein based on the weight of the composition, the contents of compounds A,B,C,D,E and is 1.25-2.10% compound A, 0.89-1.50% compound B, 2.40-3.80% compound C, 2.10-3.40% compound D, 2.74-4.60% compound E and 3.24-5.40% compound F.--

Docket No. 1624-0132P

REMARKS


The specification has been amended to provide a cross-reference to the previously filed International Application. The claims have also been amended to delete multiple dependencies and to place the application into better form for examination. Entry of the present amendment and favorable action on the above-identified application are earnestly solicited.

Attached hereto is a marked-up copy of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By   
James M. Slattery, #28,380

JMS/cqc  
1624-0132P

P.O. Box 747  
Falls Church, VA 22040-0747  
(703) 205-8000

Attachment: Version With Markings Showing Changes Made

(Rev. 01/22/01)

VERSION WITH MARKINGS SHOWING CHANGES MADE

The title has been amended to read as follows:

NOVEL GYMNEMIC ACID DERIVATIVES, PROCESS FOR THE PREPARATION THEREOF AND USE THEREOF AS MEDCINE

The specification has been amended to provide cross-referencing to the International Application.

The claims have been amended as follows:

A new claim 14 has been added.

10. (Amended) A composition of [claims 1 or 2]claim 1, which contains Gymnemic Acid derivative of formula I and/or II, wherein based on the weight of the composition, the contents of compounds A,B,C,D,E and is 1.25-2.10% compound A, 0.89-1.50% compound B, 2.40-3.80% compound C, 2.10-3.40% compound D, 2.74-4.60% compound E and 3.24-5.40% compound F.

## **New Gymnemic Acid Derivatives, their Preparation, Pharmaceutical Composition Containing them and Their Medical Use**

### **The Invention Field**

This invention relates to new Gymnemic acid derivatives, their preparation, pharmaceutical composition or extract which contains them, and their medical use, especially the use in the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation.

### **Background of technology**

A lot of study on Gymnemic Acid derivatives have been done and all of these Gymnemic acid derivatives are from the plant called Gymnema cane, which is classified as *Gymnema sylvestre*. R. Br. And in India, it has been used to treat swelling, snake venom toxin, malaria, as a diuretic or to lower blood sugar level. Yet the Gymnemic acid derivatives and their biological activity mentioned in this invention haven't been reported up to date.

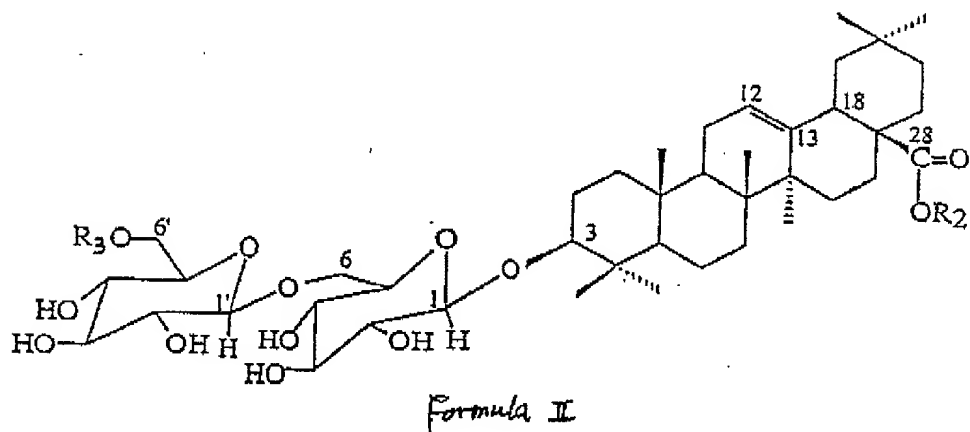
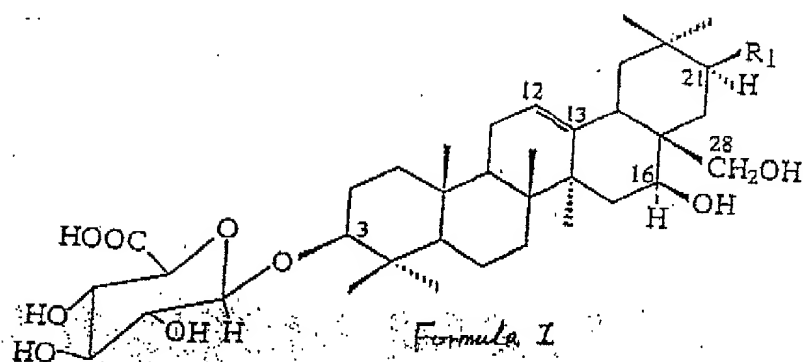
### **The object of this invention**

The object of this invention is to find new Gymnemic acid derivatives and develop their medical use.

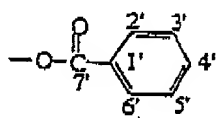
### **Description of this invention**

This inventor has found out new Gymnemic acid derivatives formula I or II and further their medical use, especially in treating hyperglycemia, hyperlipidemia and platelets aggregation. The invention is now performed based on the discovery mentioned above.

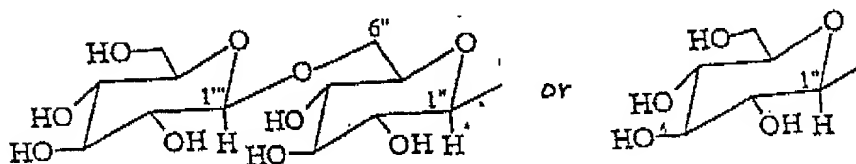
In the first part, this invention concerns Gymnemic Acid derivatives formula I or II,



wherein, R<sub>1</sub> is H or the radical represented by the following formula

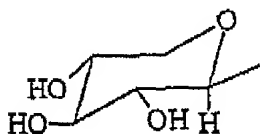


R<sub>3</sub> is H, and R<sub>2</sub> symbolizes the following radical, or

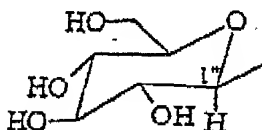




R<sub>3</sub> symbolizes the following radical,



R<sub>2</sub> is H or the following radical ,



or pharmaceutically base addition salt thereof.

The second part of this invention relates to pharmaceutical composition which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as active ingredient, pharmaceutical carrier and excipient.

The third part of the invention involves Gymnemic Acid extract, 12.5—40wt% of which is Gymnemic Acid derivative of formula I and/or II.

Another part of this invention relates to pharmaceutical composition for the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.

Another part of the invention relates to a pharmaceutical composition for

the prevention or treatment of diabetic, which includes at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, officinal carrier and excipient.

Another part of this invention relates to pharmaceutical composition for the prevention or treatment of higher blood lipid level, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.

Another part of this invention relates to pharmaceutical composition for the prevention or treatment of platelets aggregation, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.

Another part of this invention relates to the preparation of Gymnemic Acid derivative of formula I and II or pharmaceutical base addition salt thereof, which includes the following steps:

- a) extracting the plant *Gymnema cane* with ethanol under reflux and then concentrating;
- b) extracting concentrated liquid in step a) with cyclohexane , then extracting with n-butanol, concentrating to dryness under reduced pressure, and then obtaining a ointment;
- c) subjecting the ointment in step b) to silica column chromatography with elute as chloroform: methanol=90:10—50:5 or 90:10—60:40, obtaining Gymnemic acid derivative of formula I and residue;
- d) subjecting the residue in step c) to  $C_{18}$  column chromatography with elute as methal/water (20/80-40/60), obtaining Gymnemic acid derivative of formula

II;

- e) if desired, converting the obtained Gymnemic acid derivative of formula I or II into pharmaceutical base addition salt with inorganic or organic base.

Another part of this invention relates to a method of preparation of the extract containing Gymnemic Acid derivative of formula I and II which range from 12.5-40wt%, which includes the following steps:

- a) extracting *Gymnema* cane leaves with 60-95% ethanol and concentrating,  
b) extracting concentrated liquid in step a) with cyclohexane, then extracting with n-butanol, and then concentrating the extract under reduced pressure.

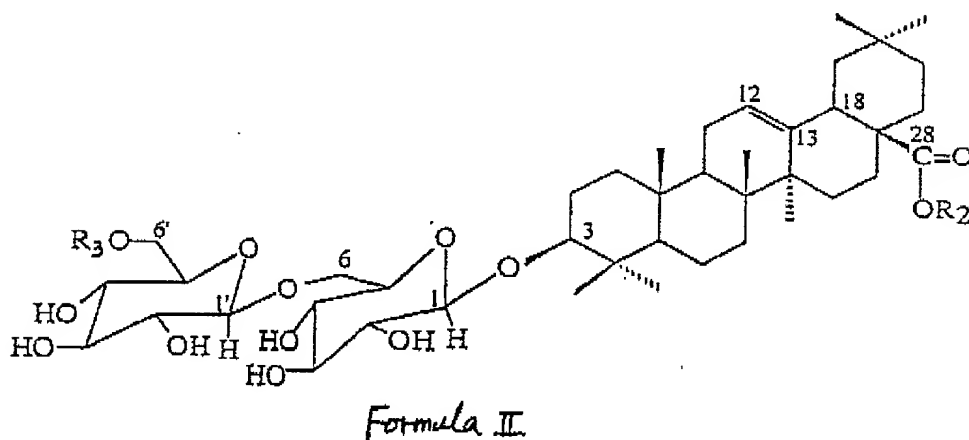
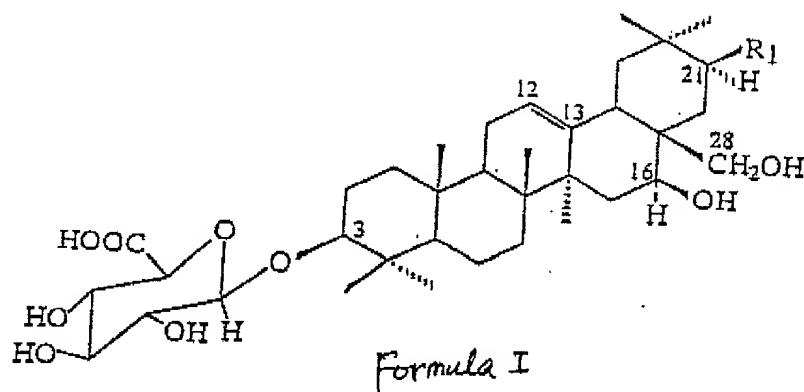
Another aspect of the invention relates to use of Gymnemic Acid derivative of formula I and II or the extract containing Gymnemic Acid derivative of formula I and II for the manufacture of medicament for the prevention or treatment of the diseases and conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation.

Finally, this invention relates to the method of preventing or treating the diseases and conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation, which includes administering prophylactic or treatment effective quantity of Gymnemic Acid derivative of formula I and II to the patient suffered from diseases or conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation.

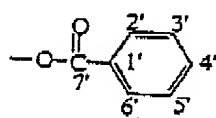
The term "patient" in the invention refers to mammal, including human being, and especially human being.

## Detailed Description of the Invention

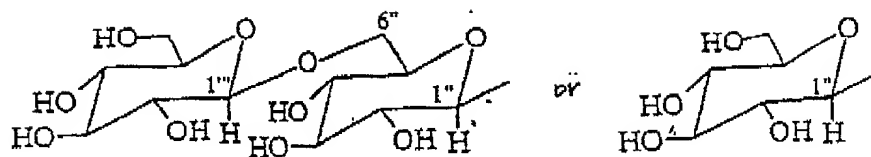
This invention relates to Gymnemic Acid derivative of formula I and II,



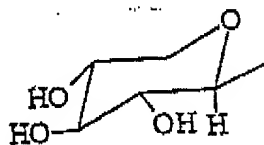
Wherein,  $R_1$  is H or the group of the following formula



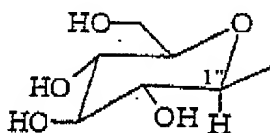
R<sub>3</sub> is H, R<sub>2</sub> is the following group, or



R<sub>3</sub> is the following group,



R<sub>2</sub> is H or the following group,

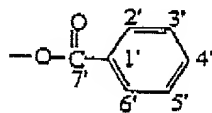


or pharmaceutical base addition salt.

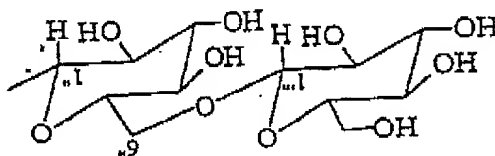
According to the present invention, pharmaceutical base addition salt of Gymnemic acid of formula I or II includes a salt formed with pharmaceutical inorganic or organic base, inorganic base, for example, includes alkali or alkali earth metal hydroxide, alkali metal or alkali earth metal carbonate or bicarbonate, alkali metal may be selected from Li, Na, K, alkali earth metal may be selected from Ba, Mg, Ca etc. The organic base, for example, may be triethyl amine etc.

According to this invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula I wherein R<sub>1</sub> is H..

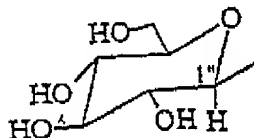
According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula I wherein  $R_1$  as the following radical.



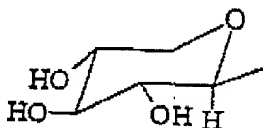
According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula II wherein  $R_3$  as H and  $R_2$  as the following radical.



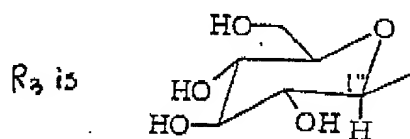
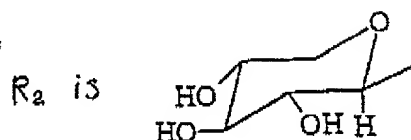
According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula II wherein  $R_3$  as H and  $R_2$  as the following radical.



According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula II wherein  $R_3$  as the following radical and  $R_2$  as H.



According to the present invention, Gymnemic acid compound prefers Gymnemic Acid compound of formula II wherein both  $R_3$  and  $R_2$  as the following radical respectively.



According to this invention, the pharmaceutical composition mentioned here contains at least one kind of Gymnemic Acid derivative of formula I and/or II and pharmaceutical carrier and excipient. For example, the pharmaceutical composition may include, for example, 1.25-2.10wt% compound A, 0.89-1.50wt% compound B, 2.40-3.80wt% compound C, 2.10-3.40wt% compound D, 2.74-4.60wt% compound E, and 3.24-5.40wt% compound F (compounds A, B, C, D, E and F as defined in examples below.). This pharmaceutical composition can be administrated by gastro intestine, parenteral or topical administration, such as oral, muscle, subcutaneous, peritoneum, vein etc. The forms of drug suitable for intestine administration are for example tablet, capsule, solution, suspension, powder, granulate etc. The forms of drug suitable for parenteral include injection solution, frozen dry powder for injection etc. The drug forms suitable for the topical are for example, ointment, cream, paste, patch, and spray. Of all these forms, oral administration is preferred while capsule is preferred in oral form. The

pharmaceutical carrier or excipient of the pharmaceutical composition includes binding agent, filling material, wet agent, desintegrate agent, surfactant, lubricating agent, dilut agent etc. If desired, colour agent, flavoring agent, solubilizer, buffer etc are also used. The diluting agents in the invention include starch, dextrin, lactose, microcellulose, silica gel, etc. And silica gel is preferred. The wetting agents includes water and ethanol, lubricating agents include talcum powder, stearic magnesium.

The pharmaceutical composition in the present invention can be produced by the known method in this art. For example, mix Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt with pharmaceutical carrier and excipient.

The dose of Gymnemic Acid derivative of formula I and II depends on many factors such as the characters and seriousness level of the disease to be prevented or treated, sex, age, weight, individual response, specific compound, administration route and times of administration. Generally the specific dose depends on the judgment of physician. Generally speaking, the dosage the pharmaceutical composition Gymnemic Acid derivative of formula I and II can be in the form of single dose and taken 1-4 times per day.

According to this invention, the or pharmaceutical base said of formula I Gymnemic Acid derivative can be prepared as follows:

- a) crushing dry leaves of *Gymnema cane*, then extracting three times with 60-95% ethanol under reflux, two hours for each, combining extracted liquid and concentrating under reduced pressure until there was no ethanol, for use;
- b) extracting the concentrated mixtures in step a) for 3 to 6 times with cyclohexane, then extracting with n-butanol, concentrating to dryness under reduced pressure, obtaining dry extract, ready for use;
- c) subjecting the dry extracts in step b) to silica gel column chromatography



with elute as mixture of chloroform and methanol at the ratio 90:10 to 60:40, obtaining derivatives of formula I,

- d) If desired, converting the derivative of formula I in step c) into pharmaceutical base salt thereof.

According to this invention, the Gymnemic Acid derivative of formula II can be prepared as follows:

- a) Crushing dry leaves of *Gymnema* cane, then extracting three times with 60-95% ethanol under reflux, two hours for each, combining extracted liquid and concentrating under reduced pressure until there was no ethanol, ready for use.
- b) extracting concentrated mixtures for 3 to 6 times with cyclohexane, then extracting with n-butanol; concentrating to dryness under reduced pressure, ready for use;
- c) mixing the dry extracts in step b) with raw silica gel; subjecting separation with thin layer chromatography of silica gel H; the mixture of chloroform and methanol at the ratio 90:10 to 50:50 as elute, subjecting the residue after elute to C<sub>18</sub> column chromatography with elute as methol/water (20:80-40:60), obtaining derivative of formula II;
- d) if desired, converting the derivative of formula II in step c) into pharmaceutical base salt thereof.

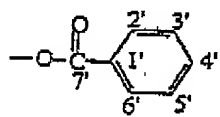
According to this invention, the extract products with 12.5-40 wt% Gymnemic Acid derivative of formula I and formula II can be prepared as follows: raw powder of *Gymnema* cane leaves were refluxed 1-4 times with 60-95% ethanol, the amount of solvent for each is 6ml/g, extract time is 1-3 hours. The extract mixtures were combined together and distilled under reduced pressure till there was no ethanol, the concentrated mixture was extracted with cyclohexane for 1-3 times, 500ml solvents was used for each time. Then the mixture was extracted for 1-3 times 500ml with n-butanol, all the extract mixtures were combined and distilled under reduced pressure to

obtain the desired product.

This invention gives a further illustration by the preparation examples and biological active experiment, but it does not means any limitation to the invention.

#### Example 1

Preparation of compound A (Gymnemic Acid derivative of formula I wherein the R<sub>1</sub> being H) and compound B (Gymnemic Acid derivative of formula I wherein the R<sub>1</sub> being group as follow)



1000g raw powder of Gymnema cane leaves were refluxed for 3 times with 60% ethanol, 6L solvents were used for each, and 2 hours for each time. The extract mixtures were combined together and distilled under reduced pressure till there was no ethanol, the concentrated mixture was extracted with 0.5L cyclohexane and butane for 3 times. All the n-butane extract mixtures were combined and distilled under reduced pressure to obtain 64.0g dry extract product. 32.0g dry extract substance was added into 60g 60-100 mesh rough silica gel, the mixture was vaporized to dryness on a water pan. 450g 200-300 mesh (m) silica gel were loaded into column by a wet method, then treated sample was added to be subjected to column separation with elute as 90:10-60:40 of mixtures of chloroform and methanol mixtures, 80mg compound A and 60mg compound B were obtained.

The physics and chemistry data of compound A and compound B were showed as follows:

#### Compound A:

Amorphous powder: mp198 – 202 °C;  $[\alpha]_{20}^D +16.0^\circ$  (c0.10, MeOH); IR $\nu_{\max}$ 3414 (OH), 1724 (COOH), 1636 (C=C), 1458, 1380, 1054cm<sup>-1</sup>; <sup>1</sup>HNMR (500MHz, pyridin - d<sub>5</sub>)  $\delta$ 0.86 (3H, s, Me), 0.95 (3H, s, Me), 1.01 (9H, s, 3 ×Me), 1.32 (3H, s, Me), 1.39 (3H, s, Me), 3.39(1H, dd, J=4.3 and 11.8Hz, H - 3 $\alpha$ ), 3.68(1H, d, J=10.5Hz, H - 28a), 4.43 (1H, d, J=10.5Hz, H - 28b), 4.68 (1H, m, H - 16 $\alpha$ ), 5.04 (1H, d, J=7.8Hz, H - 1 of gluconic acid), 5.26 (1H, brs, H - 12); <sup>13</sup>CNMR (125MHz, pyridin - d<sub>5</sub>), See table 1 and 2; FAB MSm/z 657[M+Na]<sup>+</sup>.

#### Compound B:

Amorphous; mp192 – 195 °C;  $[\alpha]_{20}^D +27.2^\circ$  (c 0.15, MeOH); IR $\nu_{\max}$ 3444 (OH), 1724, 1700, 1635 (C=C), 1457, 1388, 1280, 1074, 720cm<sup>-1</sup>; <sup>1</sup>HNMR (500MHz, pyridin)  $\delta$ 0.98 (3H, s, Me), 1.01 (3H, s, Me), 1.02 (9H, s, 3 ×Me), 1.07 (3H, s, Me), 1.30 (3H, s, Me), 1.34 (3H, s, Me), 1.36 (3H, s, Me), 3.40 (1H, dd, J=4.5 and 12.0Hz, H - 3 $\alpha$ ), 3.70 (1H, d, J=10.2Hz, H - 28a), 4.42 (1H, d, J=10.2Hz, H - 28b), 4.70(1H, m, H - 16 $\alpha$ ), 5.10(1H, d, J=7.8Hz, H - 1 of gluconic acid), 5.70 (1H, dd, J=4.7 and 12.3Hz, H - 21 $\alpha$ ), 7.47 (3H, overlap, H - 3', - 4' and - 5'), 8.25 (2H, dd, J=1.4 and 4.8Hz, H - 2' and - 6'); <sup>13</sup>CNMR (125MHz, pyridin - d<sub>5</sub>), See table 1 and 2 ; FAB MSm/z 777[M+Na]<sup>+</sup>.

Table1:  $^{13}\text{C}$ NMR data of glucoside liquid of compound A and B

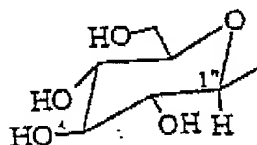
Carbon atom	Compound A	Compound B
1	38.8	38.8
2	26.6	26.6
3	89.0	89.0
4	39.5	39.6
5	55.7	55.7
6	18.4	18.4
7	32.9	33.0
8	40.1	40.1
9	47.1	47.1
10	36.7	36.7
11	23.8	23.9
12	122.6	123.1
13	143.9	142.6
14	43.8	43.7
15	36.7	36.8
16	66.6	66.4
17	41.1	43.8
18	44.4	44.2
19	47.1	47.2
20	31.1	36.0
21	34.3	75.6
22	26.2	33.3
23	28.2	28.2
24	16.9	16.9
25	15.7	15.7
26	17.0	17.0
27	27.2	27.0
28	68.9	66.8
29	33.4	29.2
30	24.1	18.8
Acyl 1'		131.6
Acyl 2'		129.9
Acyl 3'		128.9
Acyl 4'		133.2
Acyl 5'		128.9
Acyl 6'		129.9
Acyl 7'		166.3

Table 2:  $^{13}\text{C}$ NMR data of saccharic part compound A and B

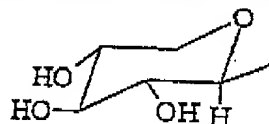
Carbon atom of C - 3	Compound A	Compound B
Glutamic acid 1	107.3	107.3
Glutamic acid 2	75.6	75.6
Glutamic acid 3	78.2	78.2
Glutamic acid 4	73.5	73.6
Glutamic acid 5	77.8	77.7
Glutamic acid 6	173.1	173.3

### Example 2:

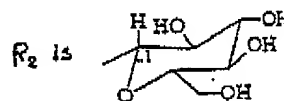
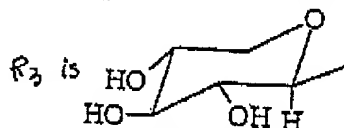
Preparation of Compound C (formula II Gymnemic Acid derivative with  $R_3$  as H and  $R_2$  as follow group),



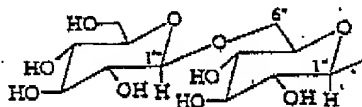
compound D (formula II Gymnemic Acid derivative with  $R_3$  as follows and  $R_2$  as H),



compound E (formula II Gymnemic Acid derivative with  $R_3$  as follow),



$R_2$  as follow and compound F (formula II Gymnemic Acid derivative with  $R_3$  as H and  $R_2$  as follow)



1000g raw powder of Gymnema cane leaves were refluxed for 3 times with 75% ethanol. 6.0L solvents were used, 2 hours for each time. The extract mixtures were combined together and distilled under reduced pressure till there was no ethanol, the condensed mixture was extracted with 0.5L cyclohexane and butane for 3 times. All the n-butane extract mixtures were gathered and distilled under reduced pressure to obtain 72.0g dry extract product. 36.0g dry extract substance was taken and added into 60g 60-100 mesh rough silica gel, the mixture was vaporized to dryness on a water pan,

400g 200-400  $\mu$  silica gel H used as thin-layer separation were loaded into column in a wet method, then treated sample was added undergoing column separation with elute as 90:10-50:50 chloroform-methanol mixtures, 130mg compound C, 115mg compound D, 160mg compound E and 195mg compound F were obtained respectively.

**The physics and chemistry data of compound C were shown as follows:**

Amorphous powder; mp 206 – 209 °C;  $[\alpha]_{20}^D - 16.0^\circ$  (c 0.11, MeOH); IR  $\nu_{\max}$  3424 (OH), 1735 (COOR), 1636 (C=C), 1457, 1034  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (400MHz, pyridin- $d_5$ )  $\delta$  0.82 (3H, s, Me), 0.87 (3H, s, Me), 0.91 (3H, s, Me), 0.97 (3H, s, Me), 1.07 (3H, s, Me), 1.20 (3H, s, Me), 1.23 (3H, s, Me), 3.17 (1H, dd,  $J=3.5$  and 10.2Hz, H-18), 3.30 (1H, d,  $J=3.9$  and 11.7Hz, H-3 $\alpha$ ), 5.37 (1H, brs, H-12),  $^{13}\text{C}$ NMR (100MHz, pyridin- $d_5$ ), See table 3 and 4; FAB MS  $m/z$  943  $[\text{M}+\text{H}]^+$ .

**The physics and chemistry data of compound D were shown as follows:**

Amorphous powder; mp 202 – 204 °C;  $[\alpha]_{20}^D - 3.2^\circ$  (c 0.15, MeOH); IR  $\nu_{\max}$  3410 (OH), 1710 (COOR), 1638 (C=C), 1458, 1036  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (400MHz, pyridin- $d_5$ )  $\delta$  0.87 (3H, s, Me), 0.91 (3H, s, Me), 0.96 (3H, s, Me), 1.02 (3H, s, Me), 1.10 (3H, s, Me), 1.24 (3H, s, Me), 1.29 (3H, s, Me), 3.30 (1H, dd,  $J=4.5$  and 11.5Hz, H-3 $\alpha$ ), 5.38 (1H, brs, H-12),  $^{13}\text{C}$ NMR (100MHz, pyridin- $d_5$ ), See table 3 and 4; FAB MS  $m/z$  935  $[\text{M}+\text{Na}]^+$ .

**The physics and chemistry data of compound E were shown as follows:**

Amorphous powder; mp 212 – 215 °C;  $[\alpha]_{20}^D - 9.6^\circ$  (c 0.20, MeOH); IR  $\nu_{\max}$  3414 (OH), 1740 (COOR), 1636 (C=C), 1460, 1364, 1044, 896  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (500MHz, pyridin- $d_5$ )  $\delta$  0.85 (3H, s, Me), 0.90 (3H, s, Me), 0.94 (3H, s, Me), 1.00 (3H, s, Me), 3.19 (1H, dd,  $J=4.0$  and 13.7Hz, H-18), 3.32 (1H, d,  $J=4.4$  and 11.7Hz, H-3 $\alpha$ ), 5.40 (1H, brs, H-12),  $^{13}\text{C}$ NMR (100MHz, pyridin- $d_5$ ), See table 3 and 4; FAB MS  $m/z$  943  $[\text{M}+\text{Na}]^+$ .

**The physics and chemistry data of compound F were shown as follows:**

Amorphous powder; mp 209 – 211 °C;  $[\alpha]_{20}^D - 12.1^\circ$  (c 0.12, MeOH); IR  $\nu_{\max}$  3424 (OH), 1734 (COOR), 1636 (C=C), 1458, 1047  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (400 MHz, pyridin- $d_5$ )  $\delta$  0.87 (3H, s, Me), 0.90 (3H, s, Me), 0.92 (3H, s, Me), 1.00 (3H, s, Me), 1.09 (3H, s, Me), 1.22 (3H, s, Me), 1.26 (3H, s, Me), 3.20 (1H, dd,  $J=3.5$  and 13.6 Hz, H-18), 3.33 (1H, d,  $J=4.4$  and 11.5 Hz, H-3 $\alpha$ ), 5.39 (1H, brs, H-12),  $^{13}\text{C}$ NMR (100 MHz, pyridin- $d_5$ ), See table 3 and 4; FAB MS  $m/z$  1127  $[\text{M}+\text{H}]^+$ .

Table3: <sup>13</sup>CNMR data of glucoside ligand of compound C-F

Carbon atom	Compound C	Compound D	Compound E	Compound F
1	38.8	38.7	38.7	38.7
2	26.6	26.7	26.7	26.7
3	88.9	89.0	89.0	89.0
4	39.4	39.5	39.5	39.5
5	55.7	55.8	55.8	55.8
6	18.4	18.3	18.5	18.5
7	33.0	33.1	33.1	33.1
8	39.8	39.9	39.9	39.9
9	47.9	48.0	48.0	48.0
10	36.9	37.0	37.0	37.0
11	23.7	23.7	23.8	23.7
12	122.9	122.8	123.0	122.9
13	144.0	144.4	144.0	144.1
14	42.0	42.1	42.1	42.1
15	28.2	28.2	28.2	28.2
16	23.3	23.4	23.4	23.4
17	46.9	46.5	47.0	47.0
18	41.6	41.9	41.7	41.7
19	46.2	46.1	46.2	46.3
20	30.7	30.9	30.8	30.8
21	33.9	34.4	34.0	34.0
22	32.5	33.1	32.5	32.5
23	28.1	28.2	28.2	28.3
24	17.0	17.0	17.0	17.0
25	15.5	15.8	15.6	15.6
26	17.4	17.3	17.5	17.5
27	26.0	26.1	26.1	26.1
28	176.4	180.2	176.5	176.5
29	33.1	33.2	33.2	33.2
30	23.6	23.7	23.7	23.7



Table 4:  $^{13}\text{C}$ NMR data of saccharic part of compound C-F

C-3	Compound C	Compound D	Compound E	Compound F
Glc1	106.9	107.0	107.0	106.9
Glc2	75.1	75.0	75.0	75.2
Glc3	78.4	78.3	78.3	78.4
Glc4	71.6	71.5	71.5	71.5
Glc5	77.0	77.0	77.0	77.0
Glc6	70.4	70.4	70.4	70.5
Glc'1	105.4	105.4	105.4	105.4
Glc'2	75.5	75.6	75.6	75.6
Glc'3	78.5	78.5	78.5	78.6
Glc'4	71.7	71.6	71.6	71.7
Glc'5	78.4	76.9	76.9	78.5
Glc'6	62.7	69.8	69.8	62.6
Xyl1		106.0	106.0	
Xyl2		74.9	74.9	
Xyl3		78.0	78.1	
Xyl4		71.1	71.1	
Xyl5		67.0	67.1	
C-28				
Glc''1	95.7		95.8	95.7
Glc''2	74.1		74.1	73.9
Glc''3	78.8		78.9	78.7
Glc''4	71.0		71.1	70.9
Glc''5	79.3		79.3	78.0
Glc''6	62.1		62.2	69.3
Glc'''1				105.3
Glc'''2				75.2
Glc'''3				78.5
Glc'''4				71.7
Glc'''5				78.4
Glc'''6				62.7

### **Biological active experiments**

#### Experiment example 1

Effect of compound B on the increasing blood sugar in rats caused by sucrose

Female SD rats fasted for 24 hours were divided into several groups randomly. Test groups are given 50,100,200mg/kg compound B, the positive-control group was given 100mg/kg phenformin, The normal group , control group and blank group were given the same amount of water, the given medicine volume was 10mg/kg, after 30 minutes each group was given saccharose 1/kg(5ml/kg) except normal group, and blood was extracted from the eyes of rats after 30, 60 and 120 minutes respectively, the content of glucose in the serum was measured.

The result was, after the rats were given saccharose for 30 ,60 minutes, the value of blood sugar increased apparently. The compound B 200mg/kg and phenformin 100mg/kg within 30 minutes can both reduce the increased value of blood sugar remarkably, and the strength of the two compounds was similar. Results see table 5.

Table 5: The effect of compound B on the increasing blood sugar in rats caused by sucrose

(  $\bar{X} \pm SD$ , n=10 )

group	dose ( mg/kg )	Value of blood sugar ( mmol/L )		
		30minutes	60 minutes	120 minutes
Normal group		3.56±0.64	4.12±0.72	3.76±0.69
control group		6.58±0.87 <sup>△△</sup>	5.93±1.27 <sup>△△</sup>	4.54±1.37
compound B	50	6.03±0.86	6.42±0.78	4.26±1.03
	100	5.12±1.29**	5.77±1.09	4.53±0.94
	200	4.43±0.72**	4.73±0.83**	4.07±0.70
phenformin	100	4.24±0.87**	4.74±0.90*	4.79±1.03

<sup>△△</sup>P<0.01, compared with normal group; \*P<0.05, \*\*P<0.01, compared with control group.

## Experiment example 2

The effect of compound B on the contents of TG, cholesterol in serum of hyperlipidemia rats

Male SD rats with the weight of 130-170g, normal group was given common food, other groups were given food having high lipid content (1%cholesterol, 10%lard, 0.3% cholic acid, 0.2% methylthio imidazole and 88.5% common forage, made into block.). In sequentially 14 days, rats fasted 12 hours were measures by reagent box method to obtain the contents of TG and cholesterol in serum. Then they were divided according to the value of blood grease into different group. The experimental group was given 50, 100. 200mg/kg compound B, the positive-control group was given clofibrate 100mg/kg, the confrol group was given water, the given medicine volume was 10ml/kg, for 10 days, each group was still given high fat forage for 5 days before given medicine, common forage was given in the later 5 days, the rats were fasted for 11 hours before being given the final administration and blood of rat were extracted to obtain the content of TG and cholesterol in serum 1 hour after being given medicine.

The results show that 10 days after rats were given forage having high grease, the contents of TG and cholesterol increased apparently, compound B 50, 100, 200mg/kg and clofibrate 100mg/kg can both reduced the contents of TG and cholesterol in blood serum of hyperlipidemia rats, compound B 200mg/kg have the same effect as 100mg/kg clofibrate in reducing hyperlipidemia, see table 6

Table 6: The effect of compound B on the contents of blood lipid in hyperlipidemia rats

( $\bar{X} \pm SD$ ,  $n=9-10$ )

group	dose (mg/kg)	TG (mmol/L)		Total cholesterol (mmol/L)	
		Before administration	After administration	Before administration	After administration
Normal group		1.02±0.22	1.04±0.15	2.43±0.41	1.99±0.47
control group		2.64±0.82	3.04±0.93	4.10±0.51 <sup>△△</sup>	4.77±0.63 <sup>△△</sup>
compound B	50	2.72±0.61	2.41±0.44	4.29±0.60	3.92±0.58**
	100	2.54±0.90	1.75±0.53**	4.02±0.59	2.94±0.66**
	200	2.72±0.76	1.37±0.40**	4.18±0.61	2.31±0.74**
clofibrate	100	2.51±0.77	2.72±0.74	4.33±0.51	2.15±0.76**

<sup>△△</sup>P<0.01, compared with normal group; \*\*P<0.01, compared with control group

### Experiment example 3

Effect of compound B on blood platelet aggregation in rabbits invitro.

Taken blood from rabbit heart with puncture, 3.8 % potassium citrate for anticoagulation (1:9), centrifuge 15 minute in 1000rpm, take upper layer as rich blood platelet plasma (prp), And then centrifuge 10 minutes with 4000rpm, take supernatant as poor blood platelet plasma (ppp). Transfer PPP200ul to nephelotube, and add into different concentration physiological brine solution 10ul of the compound B, final concentration is respectively 250,500,1000μg/ml, add physiological brine 10μl of aspirin to positive control tube ,put it into measuring cell after warming for 2 minutes in 37℃, and add into physiological brine solution10ul of ADP sodium salt with stirring, final concentration is  $1.0 \times 10^{-5}M$ . Observe the maximal aggregation ratio on PAM-1 type of blood platelet instrument within 3 minutes.

The result shows that the compound B 500,1000ug/ml and aspirin 250ug/ml obviously inhibit blood platelet from aggregating.

Table 7: Effect of compound B on blood platelet aggregation in rabbit ( $\bar{X} \pm \text{SD}$ , n=8)

Group	Final concentration	Maximal aggregation ratio (%)	Inhibition ratio
control group		$47.9 \pm 5.2$	
compound B	250	$43.6 \pm 7.0$	9.0
	500	$35.9 \pm 4.5^{**}$	25.1
	1000	$27.8 \pm 4.8^{**}$	42.0
Aspirin	250	$23.7 \pm 6.0^{**}$	50.3

$^{**}P < 0.01$ , compared with control group

#### Experiment example 4

Effect of compound F on blood sugar elevation in rat.

Male Kun Ming strain mice are divided into randomly experiment groups, they respectively take orally the compound F 50,100,2000mg/kg, positive control group respectively take orally glybenclamide 50mg/kg, blank control group and normal control group take orally the same distilled water, the volume of medicine given is 20ml/kg, lasting 7 days. They are forbidden to give feedstuff 10 hours before the last time of administration. Each group is given dextrose solution 2.5g/kg (10ml/kg) except of normal control group. Before and after 30 minute of administration of dextrose, pick blood 100ul from eyepit, measure the content of dextrose in serum according to the way of dextrose oxygenation enzyme.

Result, after mice take orally dextrose 30 minutes , blood sugar obviously rise, Both the compound F 100,200mg/kg and 50mg/kg obviously inhibit blood sugar in mice from rising. The function of the compound B 200mg/kg and glybenclamide 500mg/kg lowering blood sugar is similar, which may be seen in table 8.

Table 8

Group		Dose (mg/kg)	Value of blood sugar
		0 minute	30 minutes
Normal group		$6.20 \pm 1.01$	$6.64 \pm 1.04$
control Group		$6.55 \pm 1.16$	$13.94 \pm 3.22$
compound B	50	$6.79 \pm 1.16$	$12.01 \pm 1.88$
	100	$6.09 \pm 1.34$	$9.59 \pm 2.25^{**}$
	200	$6.42 \pm 0.99$	$9.16 \pm 1.08^{**}$
glybenclamide	50	$4.48 \pm 0.83^{**}$	$8.18 \pm 1.72^{**}$

P<0.01, compared with normal group:  $^{**}p<0.01$ , compared with control group

#### Experiment example 5

Effect of compound F on the content of triglycerides and cholesterol in the serum of hyperlipidemia rat.

Male SD rat with weight of 130-170g. The normal group is given general feedstuff, and the other groups are given high-fat (1% cholesterol, 10% pig oil, 0.3% cloic acid, 0.2 % methylthio imidazole and 88.5 % normal feedstuff are made stuff by oneself ). After the feedstuff is in run for 14 days and the mice rat is forbidden to eat for 12 hours, measure the content of triglycerides and cholesterol in rat's serum. And then the rat are grouped randomly according to blood lipid value .The experiment group is given to this compound F 50,100,200mg/kg., the ositive-control group is given orally to clofibrate of 100mg/kg, the control group is given distilled water. The volumn of administration is given 10ml/kg, lasting 10 days. Each group is given

high-fat feedstuff in the former 5days of giving drugs, and then general feedstuff in the next 5days.Fasting of 11 hours is conducted before the last time of giving drugs. After giving drugs for 1 hour, take blood and measure the content of ester and cholesterol in the blood serum..

## Result

The content of TG and cholesterol in the blood serum of rat elevates obviously after given high-fat feedstuff for 10 days.50mg/kg, 100mg/kg, 200mg/kg of compound Fand 200mg/kg clofibrate make the level of triglycerides and cholesterol in blood serum of rat with high -fat blood diseases lower. The action 200mg/kg of the compound F is the similar as to that of 100mg/kg of clofibrate in the function of lowering blood fat.(table 9)

Table 9:the effect of compound B on the content of blood fat of rat with high-fat blood disease. ( $\bar{X} \pm SD$ , n=9—10)

group	dose (mg/kg)	triglycerides (mmol/L)		Total cholesterin (mmol/L)	
		Before administration	After administration	Before administration	After administration
Normal group		1.02±0.22	1.04±0.15	2.43±0.41	1.99±0.47
Control group		2.64±0.82	3.04±0.93	4.10±0.51 <sup>△△</sup>	4.77±0.63 <sup>△△</sup>
compound B	50	2.72±0.61	2.41±0.44	4.29±0.60	3.92±0.58**
	100	2.54±0.90	1.75±0.53**	4.02±0.59	2.94±0.66**
	200	2.72±0.76	1.37±0.40**	4.18±0.61	2.31±0.74**
clofibrate	100	2.51±0.77	2.72±0.74	4.33±0.51	2.15±0.76**

<sup>△△</sup>P<0.01, (compared with normalgroup); \*\*P<0.01(compared with control group)

## Experimental example 6 effect of compound B affects on blood platelet aggregation in rabbit.

Take blood from rabbit heart by puncturing,3.8% of potassium citrate for

anticoagulation (1:9), centrifuging 15 minute in 1000rpm, take the upper layer as blood platelet rich plasma(prp), And then centrifuga 10 minute in 4000rpm, take supernatant as blood platelet poor plasma(ppp). the final concentration of compound B is revetively 250,500,1000  $\mu$  g/ml, final concentration is respectively 250,500,1000ug/ml, add 10ul of physical brine of aspirin to the positive-control tube to final concentration of 250  $\mu$  g/ml, add 10ul of physical brine to control tube to the final concentration of 250  $\mu$  g/ml. Observe the maximal aggregation ratio on PAM-1 type instrument of blood platelet aggregation within 3 minute.

The result shows that 500,1000 $\mu$ g/ml of the compound F and aspirin 250 $\mu$ g/ml obviously inhibit the aggregation of blood platelet.

Table 10: the effect of the compound F on aggregation of rabbit's blood platelets in vitro.

group	Final concentration ( $\mu$ g/ml)	Maximal aggregation rate (%)	Inhibition rate (%)
control		47.9 $\pm$ 5.2	
compound F	250	43.6 $\pm$ 7.0	9.0
	500	35.9 $\pm$ 4.5**	25.1
	1000	27.8 $\pm$ 4.8**	42.0
aspirin	250	23.7 $\pm$ 6.0**	50.3

\*\*P<0.01(compared with the control)

### Experimental example 7

Effect of compound B on blood sugar in normal mice.

Male Kun Ming strain mice are divided into randomly experiment groups, they respectively take orally the compound B 50,100,2000mg/kg, positive control group respectively take orally tolbutol 100mg/kg, blank control group takes orally the same distilled water, the volume of medicine given is 20ml/kg, lasting 14 days. They are administrated to test drug (provided that they are pre-forbidden to give food 5 hrs before administration) after the days 1, 3, 7, 14 of administration. After 3hrs of administration, pick



blood 10ul from eyepit, measure the content of dextrose in serum according to the way of reagent box.

Result shows that compound B 50, 100, 200mg/kg for continucly administration 14 days has no obvious effect on blood sgar of normal onice, but tolbutol starting from day 3 of administration show obvious effect for lowering the blood sugar of normal mice. Result is also seen in table 11.

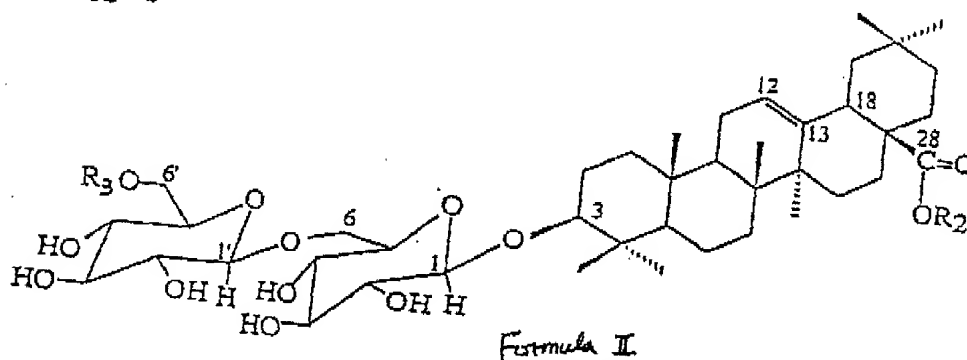
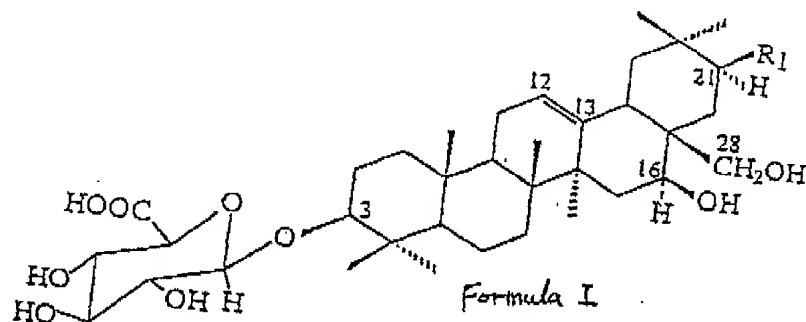
Table 11, Effect of compound B on blood sugar in normal mice.  
( $\bar{X} \pm SD$ , n=10)

Group	Dose (mg/kg)	Value of blood sugar			
		1	3	7	14 (day)
control Group		5.21 $\pm$ 1.10	7.10 $\pm$ 1.30	8.56 $\pm$ 0.74	7.52 $\pm$ 1.29
compound B	50	5.84 $\pm$ 0.94	7.56 $\pm$ 0.92	8.51 $\pm$ 1.06	8.27 $\pm$ 0.66
	100	6.48 $\pm$ 1.28	7.73 $\pm$ 2.26	8.71 $\pm$ 0.97	7.45 $\pm$ 1.59
	200	6.41 $\pm$ 1.04	6.28 $\pm$ 1.19	8.46 $\pm$ 0.88	7.86 $\pm$ 1.56
tolbutol	100	6.48 $\pm$ 1.18	5.22 $\pm$ 0.80**	6.62 $\pm$ 0.96	5.75 $\pm$ 1.02

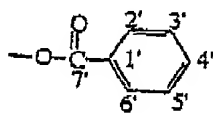
P<0.01, compared with normal group: \*\*p<0.01, compared with control group

## What is claimed

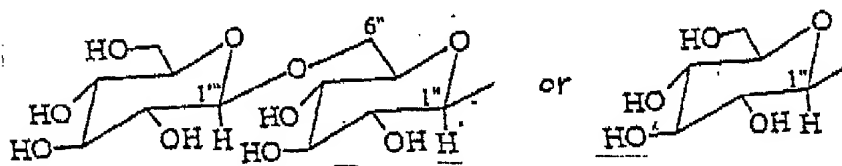
1. Gymnemic Acid derivative of general formula I or general formula II,



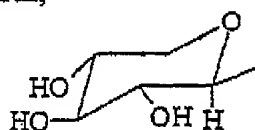
wherein,  $R_1$  is H or the radical represented by the following formula



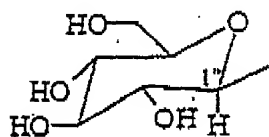
$R_3$  is H, and  $R_2$  symbolizes the following radical, or



$R_3$  symbolizes the following radical,



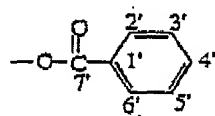
R<sub>2</sub> is H or the following radical ,



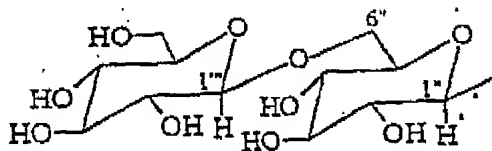
or pharmaceutically base addition salt thereof.

2. Gymnemic Acid derivatives of claim 1, wherein R<sub>1</sub> in formula I is hydrogen.

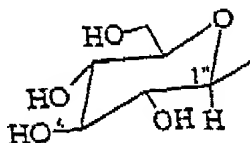
3. Gymnemic Acid derivatives of claim 1, wherein R<sub>1</sub> in formula I is a group of the formula:



4. Gymnemic Acid derivatives of claim 1, wherein R<sub>3</sub> in formula II is hydrogen, R<sub>2</sub> is group of formula:

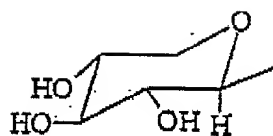


5. Gymnemic Acid derivatives of claim 1, wherein R<sub>3</sub> in formula II is hydrogen, R<sub>2</sub> is group of formula:

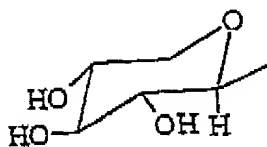


6. Gymnemic Acid derivatives of claim 1, wherein R<sub>2</sub> in formula II is hydrogen,

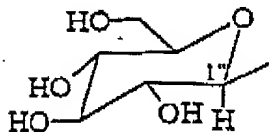
R<sub>3</sub> is group of formula:



7. Gymnemic Acid derivatives of claim 1, wherein R<sub>3</sub> in formula II is group of formula



R<sub>2</sub> is group of formula:



8. Pharmaceutical composition which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as active ingredient, pharmaceutical carrier and excipient.
9. Pharmaceutical composition for the prevention or treatment of the diseases associated with hyperglycemia, hyperlipidemia and platelets aggregation, which contains at least one kind of Gymnemic Acid derivative of formula I and/or II or pharmaceutical base addition salt thereof as a active ingredient, pharmaceutical carrier and excipient.
10. A composition of claims 1 or 2, which contains Gymnemic Acid derivative of formula I and/or II, wherein based on the weight of the composition, the content of compounds A, B, C, D, E and is 1.25-2.10% compound A,

0.89-1.50% compound B, 2.40-3.80% compound C, 2.10-3.40% compound D, 2.74-4.60% compound E and 3.24-5.40% compound F.

11. A extract of *Gymnema sylevestre*.R.Br which contains 12.5-40wt% Gymnemic acid derivatives of formula I and formula II.

12. Use of Gymnemic Acid derivative of formula I and II or the extract containing Gymnemic Acid derivative of formula I and II for the manufacture of medicament for the prevention or treatment of the diseases and conditions associated with hyperglycemia, hyperlipidemia and platelets aggregation.

13. A method of the preparation of Gymnemic Acid derivative of formula I and II or pharmaceutical base addition salt thereof, which includes the following steps:

c) extracting the plant *Gymnema* cane with ethanol under reflux and then concentrating;

d) extracting concentrated liquid in step a) with cyclohexane, then extracting with n-butanol, concentrating to dryness under reduced pressure, and then obtaining a ointment;

c) subjecting the ointment in step b) to silica column chromatography with elute as chloroform: methanol=90:10—50:5 or 90:10—60:40, obtaining Gymnemic acid derivative of formula I and residue;

d) subjecting the residue in step c) to  $C_{18}$  column chromatography with elute as methal/water (20/80-40/60), obtaining Gymnemic acid derivative of formula II;

e) if desired, converting the obtained Gymnemic acid derivative of formula I or II into pharmaceutical base addition salt with inorganic or organic base.

## Declaration and Power of Attorney for Patent Application

## 專利申請聲明及委託書

Chinese Language Declaration

## 中文聲明

作為下述發明者，我在此宣告：

As a below named inventor, I hereby declare that:

我的住址、郵局地址和國籍均列在我名下，

My residence, post office address and citizenship are as stated next to my name.

我相信我是首創的、第一個和唯一的發明者(如只列出一人姓名)或是首創的、首位共同發明者(如列出數人姓名)。我提出作為專利申請權利要求的題目如下

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

NOVEL GYMNEMIC ACID DERIVATIVES, PROCESS  
FOR THE PREPARATION THEREOF AND USE  
THEREOF AS MEDICINE

如不在下面小方格中打叉則須將說明書附此：

the specification of which is attached hereto unless the following box is checked:

☐ 以美國申請號碼或PCT國際申請號碼  
立案于  
修正于(如適用)☒ was filed on August 10, 2001  
as United States Application Number or PCT  
International Application Number  
09/913,322 and was amended on  
(if applicable).

我在此聲明我已閱畢并理解上述說明書的內容，包括上述任何修正案所修正的權利要求。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

按照聯邦法規第三十七節第一、五六條，我有責任提供支持專利權的實質性資料。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

Chinese Language Declaration

我申請享受按照美國法規第三十五節第一百一十九條(a)-(d)項或第365條(b)項列出的以下任何外國專利申請書或發明者證書或第365條(a)項列出任何PCT國際申請指定至少在美國以外的任何一個國家的外國優先權，並確認下列方格內打記號，具有優先權申請前立案日期的、任何外國專利申請書或發明者證書或是PCT國際申請書。

國外優先申請書

99100721.2	CHINA
99100722.0	CHINA
(號碼) (Number)	(國名) (Country)
99102823.6	CHINA
(號碼) (Number)	(國名) (Country)
99103588.7	CHINA
(號碼) (Number)	(國名) (Country)

我申請享受被美國法規第35節119(e)列出的以下任何美國臨時申請書的利益。

(申請順序號碼) (Application No.)	(申請日期) (Filing Date)
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我申請享受按照美國法規第三十五節一百二十條或365條(c)項列出任何PCT國際申請所指定的美國列出的以下任何美國申請書的利益，如果此申請書中提出的每項權利要求的題目未按美國法規或是PCT國際申請第三十五節第一百二十條第一段的要求在以前的美國申請書中披露，則我有責任按照聯邦法規第三十七節第一、五六(甲)條提供支持專利權的實質性資料，這一法規條文生效于以前申請的立案日期之後，但在美國或PCT國際申請立案日期之前。

(申請順序號碼) (Application No.)	(申請日期) (Filing Date)
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(申請順序號碼) (Application No.)	(申請日期) (Filing Date)
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我在此聲明根據我所知而作的所有聲明都真實無誤，所有有關資料和信息的聲明也真實無誤；我還知道，按照美國法規第十八節第一千零一項，任何蓄意偽造的聲明都將受到罰款或監禁，或同時受到兩種懲罰。這類蓄意偽造的聲明將危及此申請書或任何已頒發專利的效力。

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

不要求優先權  
Priority Not Claimed

11 February 1999	<input type="checkbox"/>
11 February 1999	<input type="checkbox"/>
(申請日/月/年) (Day/Month/Year Filed)	
12 March 1999	<input type="checkbox"/>
(申請日/月/年) (Day/Month/Year Filed)	
15 April 1999	<input type="checkbox"/>
(申請日/月/年) (Day/Month/Year Filed)	

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(申請順序號碼) (Application No.)	(申請日期) (Filing Date)
-------------------------------	-------------------------

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(狀況) (已獲專利權、申請中、取消)	(Status) (patented, pending, abandoned)
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(狀況) (已獲專利權、申請中、取消)	(Status) (patented, pending, abandoned)
------------------------	---

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issued thereon.

## 委託書：

以列名發明者的身份，我在此指定下列律師和/或代理人執行此申請並從事與專利商標公署有關的所有業務（列出姓名和註冊號碼）：

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: (list name and registration number)

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MARC S. WEINER (Reg. No. 32,181)  
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第二個發明者簽字	日期	Second inventor's signature	Yue Dai Date October. 17. 2001
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國籍		Citizenship	Chinese
郵局地址		Post Office Address	Pharmacology Dept, China Pharmaceutical University No. 1, Shennong Road, Nanjing, Jiangsu Province 210038 CHINA

（第三個和其他共同發明者需提供同樣資料和簽字。）

(Supply information and signature for third and subsequent joint inventors.)



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發明者簽字	日期	Inventor's signature	Date
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國籍		Citizenship Chinese	
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發明者簽字	日期	Inventor's signature	Date
		Xingxiang Zhu	October 17, 2001
住址		Residence Nanjing, CHINA CNX	
國籍		Citizenship Chinese	
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		Shouxun ZHAO	October 17, 2001
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國籍		Citizenship Chinese	
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第六個或唯一的發明者全名 (如有)		Full name of sixth joint inventor, if any	
發明者簽字	日期	Inventor's signature	Date
住址		Residence	
國籍		Citizenship	
郵局地址		Post Office Address	